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COMPLEMENT FIXATION IN INFLUENZA *

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It was thought that it might be of interest to examine the serum of influenza patients for immune bodies that would bind complement in the presence of antigens made from organisms isolated in this disease. The results of cultures reported from different places varied so greatly that it was decided to obtain both serum and bacterial strains for antigens from several sources.

Convalescent serum was obtained from Michael Reese Hospital, Wesley Memorial Hospital and the University of Chicago, Chicago; from Camp Green, N. C.; from Camp Grant, Ill., and from the U. S. Naval Hospital, the U. S. Naval Medical School and the Hygienic Laboratory, Washington, D. C. The serum of four convalescent scarlet fever patients, of three convalescent diphtheria patients and from seven persons who had not had influenza, was used as controls.

Ten of the convalescent serums from Michael Reese Hospital were from moderately ill, uncomplicated cases of influenza, four from patients with influenza complicated by pneumonia. Of the latter, serum 3 came from a patient who had a very severe illness extending over 5 weeks. Other complicated cases were only moderately ill. In all cases, the serum was obtained from 3-7 seven days after the temperature had reached normal. The serum from Camp Green was obtained from a patient who several weeks before had a typical attack of influenza. Five serums from Wesley Memorial Hospital were from uncomplicated influenza and two from influenza and pneumonia. Details as to the nature of the influenzal attack were not obtained in the other cases.

As antigens, streptococci from blood cultures made in the Michael Reese Hospital were used. The streptococci were of two kinds:

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(1) A nonhemolytic, green-producing diplococcus; the colonies, which appeared in 24 hours, were moist, elevated, with slightly irregular margins, and produced no hemolysis during the 10 days in which they were observed; microscopically, it was a fair-sized, gram-positive diplococcus in pairs and chains. (2) A small gram-positive coccus in pairs and chains which after 24-48 hours appeared as minute green colonies and after 48-72 hours formed a very slight zone of almost complete hemolysis. Both varieties fermented levulose and dextrose, but not inulin nor mannite, and were not soluble in bile. Antigens were made from two strains of the first and four strains of the second. Other bacteria used for antigens were as follows: Influenza bacilli isolated from cases of influenza in Chicago and New York City; (1) atypical viridans streptococci isolated from cases of influenza by the late Capt. George Mathers at Camp Meade, Md.; similar cocci isolated by Dr. Ruth Tunnicliff from brains of persons dying with influenzal pneumonia in Chicago; pneumococci of different types; typical hemolytic streptococcus from a brain abscess; also *B. mucosus* and *M. catarrhalis*.

The serums were heated for 30 minutes at 56 C. and used undiluted with the exception of the specimens from the U. S. Naval Medical School, Washington, D. C., which contained a small amount of sodium citrate.

Unheated bacterial suspensions were used as antigens. In addition to the bacterial antigens two antigens were made from the sputum of patients severely ill with influenza; the sputum was ground with sterile sand and salt solution, and part of the suspension filtered through a Berkefeld filter; cultures of the filtrate were sterile; the other part was centrifugalized slowly to remove the larger particles, the supernatant fluid heated and used as antigen. In order to have sufficient serum for many antigens, only two antigen dilutions were used, $\frac{1}{4}$ and $\frac{1}{40}$ of the anticomplementary unit.

The antishoop rabbit system was employed in $\frac{1}{10}$ the volume of the original Wassermann test. Serum, antigen and complement (fresh guinea-pig serum, 2 units) were incubated at 37 C. for 1 hour when 2 units of previously titrated antishoop amboceptor and sheep corpuscles (5% suspension) were added, and the whole incubated for 1 hour. The customary antigen, serum and hemolytic control tests were made each time.

Source of Serum		Atypical Streptococci of the Viridans Group													
		Michael Reese Hospital						Camp Meade							
		Slightly Hemolytic				Nonhemolytic									
		I	II	III	IV	I	II	I	II	III	IV	V	VI	VII	VIII
Michael Reese Hospital—Influenza															
Case 1.....	0	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
2.....	0	++++	0	++	++++	++++	++	++++	++++	++++	++
3.....	0	++++	++++	++++	++	++++	++++	++++	++
4.....	0	0	0	++++	++++	++++	++++	++++	++++	++++	++
5.....	0	0	0	++++	++++	+	++++	++++	++++	++++	++
6.....	0	0	0	0	++	++++	+	++	++++	++++	++	++
7.....	0	0	0	0	++	++++	+	++	++++	++++	++	++
8.....	++++	0	0	0	++++	++	+	++	++++	++++	+	++
9.....	+	0	0	0	++++	++	++	++	++++	++++	++	++
10.....	0	0	0	0	0	++	0	0	++++	++++	++	++
Influenza and Pneumonia															
Case 1.....	0	0	0	0	++	++	+	++	++++	++++	0	0
2.....	0	0	0	0	+	++	+	++	++++	++++	0	++
3.....	0	0	0	+	++	+	0	++++	++++	0	++
4.....	+	0	0	0	++++	++++	0	0	++++	++++	0	++
5.....	0	++	++++	0	0	++++	0	0	0	0	++
6.....	++++	++	++++	++++	++	++	++	+	0	0	++++
7.....	0	++	0	++	+	++++	+	+	+	++	++++
Wesley Memorial Hospital—Influenza															
Case 1.....	0	0	0	++	++	+	++	++++	++++	0	0
2.....	0	0	0	+	++	+	++	++++	++++	0	++
3.....	+	++	+	0	++	0	0	0	0
4.....	++	0	++	0	0	0	0	0	0	0
5.....	+	++++
Influenza and Pneumonia															
Case 1.....	0	0	0	++	+++	+	0	++++	+++	0	++
2.....	++	0	0	0	+	+	0	0	++++	++	0	++
Camp Greene—Influenza.....		0	0	0	++	++	+	0	++++	++	+++	++
Camp Grant—Influenza															
Case 1.....	0	0	+	+++	0	0	+	+	++	+
2.....	0	0	+	0	0	+	++	++	0	0
University of Chicago—Influenza															
Case 1.....	0	0	0	++
2.....	0	0	0	0	++
3.....	0	0	0	0	0
4.....	0	0	0	0	0
5.....	0	0	0	0	0
6.....	0	0	0	0	0
7.....	+	0	0	+++	++
8.....	+	+	0	+++	++
U. S. Naval Medical School—Influenza															
Case 1.....	0	+	+	0	++++	+	++	++	0	++	++
2.....	+++	0	+	0	++++	+	+++	++	++	++	++
3.....	++	0	0	+	++++	+	++++	+	0	++	++
4.....	++	0	+	0	++++	+	++++	0	++	0	++
5.....	++++	+	+	+	++++	+	++	++++	++	++	+
6.....	++++	0	+	+	++++	+	+	++	++	++	++
7.....	++	0	+	0	++++	+	++++	0	++	++	++
8.....	++++	0	+	0	++++	+	++++	0	++	++	0
9.....	++++	0	+	0	++++	+	++	+	0	0	0
10.....	0	0	0	0	+	0	+++	+	0	++	+
11.....	0	0	+	0	0	+	0	0	++	0	++
12.....	0	+	+	0	0	0	+	0	0	++	0
13.....	0	+	+	0	++	0	+	0	0	0	+
14.....	0	+	++++	0	0	0	+++	0	++	++	+
15.....	+	+	+	++++	0	0	0	0	0	++++	+
16.....	0	+	0	0	0	+	+	++	0	++
17.....	++	+++	+	+++	+	+++	0	0	+	++	+++
18.....	+	+	++	++	+	++	+	0	+	+++	++
19.....	0	++	++	++	0	+++	+	0	++	+++	+++
20.....	0	++	0	0	++	++	++	++	+++	+++	+
21.....	0	++	++	0	++++	++	++	+++	+++	+++	++
22.....	0	++	+++	+++	+++	++	+++	++	+++	+++	++
23.....	0	+	+++	+++	+++	+++	+++	+++	+++	+++	+++
24.....	0	0	0	0	+	0	0	+	++	+++	++
25.....	0	0	0	0	+	+	0	+	++++	+++	+++
26.....	0	0	0	0	0	0	+	0	0	+++	+++
27.....	0	0	0	0	0	0	+	0	0	++	++
Hygienic Laboratory, Washington, D. C.—Influenza															
Case 1.....	0	0	0	0	+	+	0	+	+	++	0
2.....	0	++	0	0	0	0	0	0	0	++	++
3.....	0	+	0	0	++	++	0	+	++	+	++
4.....	0	++	0	++	++	+	+	+	0	+	++

[illegible]

Influenza Case														
1.....	0	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
2.....	0	0	++++	0	++	++++	++++	++	++++	++++	++++	++++
3.....	0	++++	0	++++	++++	++++	++	++++	++++	++++	++++
4.....	0	0	0	++++	++++	++++	++++	++++	++++	++++	++++
5.....	0	0	0	++++	++++	++++	++++	++++	++++	++++	++++
6.....	0	0	0	0	++	++++	+	++++	++++	++++	++	++
7.....	0	0	0	0	++	++++	+	++++	++++	++++	++	++
8.....	++++	0	0	0	++++	++	+	++++	++++	++++	+	++
9.....	+	0	0	0	++++	++	++	++++	++++	++++	+	++
10.....	0	0	0	0	0	++	0	++++	++++	++++	++	++
Influenza and Pneumonia Case														
1.....	0	0	0	0	++	++	+	++	++++	++++	0	0
2.....	0	0	0	0	+	++	+	++	++++	++++	0	++
3.....	0	0	0	+	++	+	0	++++	++++	0	++
4.....	+	0	0	0	++	++	0	0	++++	++++	0	++
5.....	0	++	++++	0	0	++	0	0	0	0	++
6.....	++++	++	++++	++++	++	++	+	0	0	0	++
7.....	0	++	0	++	+	++++	+	+	+	+	++
Wesley Memorial Hospital—Influenza Case														
1.....	0	0	0	++	++	+	++	++++	++++	0	0
2.....	0	0	0	+	++	+	++	++++	++++	0	++
3.....	+	++	+	0	+	0	0	0	0
4.....	++	0	++	0	0	0	0	0	0	0
5.....	+	++++
Influenza and Pneumonia Case														
1.....	0	0	0	++	+++	+	0	++++	+++	0	++
2.....	++	0	0	0	+	+	0	0	++++	++	0	++
Camp Greene—Influenza.....														
.....	0	0	0	++	++	+	0	++++	++	+++	++
Camp Grant—Influenza Case														
1.....	0	0	+	+++	0	0	+	+	++	+
2.....	0	0	+	0	0	+	++	++	0	0
University of Chicago—Influenza Case														
1.....	0	0	0	0	++
2.....	0	0	0	0	0	++
3.....	0	0	0	0	0	0
4.....	0	0	0	0	0	0
5.....	0	0	0	0	0	0
6.....	0	0	0	0	0	0
7.....	+	0	0	0	+++	++
8.....	+	+	0	0	+++	++
U. S. Naval Medical School—Influenza Case														
1.....	0	+	+	0	++++	+	++	++	0	++	++
2.....	+++	0	+	0	++++	+	+++	++	++	++	++
3.....	++	0	0	+	++++	+	++	+	0	++	++
4.....	++	0	+	0	++++	+	+++	0	++	0	++
5.....	+++	+	+	+	++++	+	++	++++	++	++	+
6.....	+++	0	+	+	++++	+	+	++	++	++	++
7.....	++	0	+	0	++++	+	+++	0	++	++	++
8.....	+++	0	+	0	++++	+	+++	0	++	++	0
9.....	+++	0	+	0	++++	+	++	+	0	0	0
10.....	0	0	0	0	+	+	+++	+	0	++	+
11.....	0	0	+	0	0	0	0	++	0	++	+
12.....	0	+	+	0	0	0	+	0	0	++	0
13.....	0	+	+	0	++	0	+	0	0	0	+
14.....	0	+	+++	0	0	0	+++	0	++	++	+
15.....	+	+	+	+++	0	0	0	0	+++	+
16.....	+	+	0	0	0	+	+	0	++	+
17.....	++	+++	+	+++	+	0	0	+	++	+++
18.....	+	+	++	++	+	0	+	+++	+++	+++
19.....	0	++	++	++	0	++	+	+++	+++	+++
20.....	0	++	0	0	++	++	++	+++	+++	+
21.....	0	++	++	0	+++	++	++	+++	+++	++
22.....	0	++	+++	+++	++	+++	++	+++	+++	++
23.....	0	+	+++	+++	++	+++	+++	+++	+++	+++
24.....	0	0	0	0	0	0	+	+++	+++	++
25.....	0	0	0	0	+	0	+	+++	+++	+++
26.....	0	0	0	0	0	+	0	0	+++	+++
27.....	0	0	0	0	0	+	0	0	++	++
Hygienic Laboratory, Washington, D. C.—Influenza Case														
1.....	0	0	0	+	0	0	+	+	++	+
2.....	0	++	0	0	0	0	0	0	0	++	++
3.....	0	+	0	0	++	++	0	+	++	+	++
4.....	0	++	0	+	++	0	+	+	+	++	++
Scarlet Fever Case														
1.....	0	0	0	0	0	0	0	0	0	0	0
2.....	0	0	0	0	0	0	0	0	0	0	0
3.....	0	0	0	0	0	0	0	0	0	0	0
4.....	0	0	0	0	0	0	0	0	0	0	0
Diphtheria Case														
1.....	0	0	0	0	0	0	0	0	0	0	0
2.....	0	0	0	0	0	0	0	0	0	0	0
3.....	0	0	0	0	0	0	0	0	0	0	0
4.....	0	0	0	0	0	0	0	0	0	0	0
Normal Persons Case														
1.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0

++++ = complete inhibition of hemolysis

+++ = almost complete inhibition of hemolysis.

++ = partial

[illegible]

[illegible]

In some instances, especially when citrated serum was used, it was necessary to place the test in the icebox over night before a final reading could be made.

A negative Wassermann was obtained in the case of each serum used in the experiments.

The results which are given in tables 1 and 2 show that the antigens from Camp Meade and similar strains of streptococci of the viridans group were the only ones that fixed complement with any degree of constancy. One hundred forty-one serums of convalescent patients were tested with the Michael Reese atypical *Streptococcus viridans* (slight hemolysis) antigen. In 40% of the tests there was some degree of fixation. With the other Michael Reese atypical *Streptococcus viridans* (no hemolysis) there was fixation in 32% of 78 tests. The Camp Meade strain was used in 484 tests, 70% of which gave fixation. With the 176 tests made with the brain antigens, fixation occurred in 42%. Filtered sputum (52 tests) and washed sputum (19 tests) gave only 20 and 21% positive fixations, respectively. With various influenza antigens 240 tests were made with fixation in 20%. Of the 281 tests with pneumococcus antigen 15% fixed complement. Antigens from hemolytic streptococcus and from *B. mucosus* gave no fixation. Three serums gave partial inhibition of hemolysis with *M. catarrhalis*, when it was used as antigen. The scarlet fever serum fixed complement in five instances and the diphtheria serum in three. The seven normal serums used as controls gave uniformly negative results.

Serum was collected on several successive days from 12 patients. The collection of serum was started on the day the patient entered the hospital, which in most cases was early in the disease. These serums were tested with Camp Meade strain 2, which had given good fixation in previous tests, and also when the amount of serum permitted with influenza bacillus strain 101. The results (table 3) show that there was considerable fixation with the Camp Meade antigen, and that apparently little relation could be made out between the degree of fixation and the stage of the disease in which the serum had been obtained. In 12 cases only a very small amount of serum was collected; ten of the twelve specimens gave definite fixation with Camp Meade strain 2.

TABLE 2
PERCENTAGES OF FIXATION AS SHOWN BY TABLE 1

Antigens	No. of Tests with Influenza Serum	Fixation	No. of Tests with Searlet Fever Serum	Fixation	No. of Tests with Diphtheria Serum	Fixation	No. of Tests with Normal Serum	Fixation
Atypical streptococci viridans group:								
Michael Reese Hospital (1).....	157	40%	8	0	6	0	28	0
Michael Reese Hospital (2).....	78	32%	8	0	6	0	14	0
Camp Meade (Captain Mathers)	484	70%	32	0	24	0	63	0
Brain (Dr. Tunncliffe).....	176	42%	20	5%	15	3%	35	0
Filtered sputum.....	52	20%	4	0	2	0	7	0
Washed sputum.....	19	21%	0	0	0	0	7	0
Influenza bacillus.....	253	20%	20	0	15	0	49	0
Pneumococcus.....	281	15%	24	0	18	0	42	0
Hemolytic streptococcus.....	36	3%	4	0	3	0	7	0
B. mucosus.....	21	0%	0	0	0	0	7	0
M. catarrhalis.....	33	15%	4	0	3	0	7	0

TABLE 3
TESTS FOR COMPLEMENT FIXATION WITH SERUMS COLLECTED ON DIFFERENT DAYS OF THE ATTACK

Case and Dates	Camp Meade Strain 2	Influenza Bacillus Strain 101	Case and Dates	Camp Meade Strain 2	Influenza Bacillus Strain 101
1 10/26	+++	0	9 11/11	0	0
10/27	+++	0	11/12	+	0
10/28	0	0	11/13	+	0
10/29	++	0	11/14	++	0
	++	0	10 11/15	++	0
2 10/26	0	0	11/16	+++	0
10/27	++	0	11 11/ 5	++	0
10/28	0	0	11/ 6	+++	0
10/29	++	0	11/10	++	0
3 10/25	++	0		+	0
10/26	++	0		++	0
10/27	++	0	12 11/ 7	++	0
10/28	0	0	11/ 8	++++	0
10/29	++	0	11/10	++	0
	0	0	13 11/ 9	+++	0
4 10/16	0	0	14 11/ 9	++++	0
	++	0	15 11/ 9	++	0
5 10/15	0	0	16 11/ 9	+++	0
	++	0	17 11/ 9	++	0
6 10/13	0	0	18 11/25	0	0
10/14	0	0	19 11/25	0	0
7 10/15	0	0	20 10/29	++	0
	++	0	21	++	0
8 11/ 4	0	0	22	++	0
11/ 7	0	0	23	++++	0
11/ 8	0	0	24	+	0
11/10	0	0			

SUMMARY

The outstanding feature of this work on complement fixation with influenza serum is the large number of positive results with certain strains of the viridans group of streptococci isolated from cases of influenza at Camp Meade and in Chicago. The evidence indicates that such organisms probably played an important part in the morbid process even in other places. Serum from influenza patients in several different places appears to have acquired similar new properties.